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JOURNAL ARTICLE

Nervous-system specific proteins as biochemical indicators of neurotoxicity

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Recent advances in neuroimmunology and protein purification methodology have led to the identification of nervous-system specific proteins. Their intimate relationship to the cellular and functional heterogeneity of the nervous system, makes these proteins ideal biochemical markers for the detection and characterization of neurotoxicity.

Although biochemical methodology has been applied to the delineation of molecular mechanisms associated with specific neurotoxic responses, this approach has not resulted in the identification of broadly applicable chemical correlates of neurotoxicity. This failure is not unexpected since most biochemical techniques chosen to detect and quantify neurotoxic effects of environmental agents evolved from investigations that established the biochemical basis of neuropharmacology.

Most tests that predict or reflect drug action on the nervous-system were designed to measure effects which (1) are restricted to specific types of neurons, (2) are reversible in nature, (3) are short lasting and (4) correlate with known actions of pharmacological agents. Thus, when applied to neurotoxicology, testing strategies predicated on procedures employed in neuropharmacological research may prove useful for assessing the effects of specific neurotoxicants on neuronal processes sensitive to pharmacological intervention. It is doubtful, however, that this approach will result in the identification of biochemical indicators with which to monitor or predict general neurotoxic responses. This is due to the fact that, unlike effects of pharmacological agents at therapeutic dosages, exposure to neurotoxic substances is often characterized by slowly reversible or irreversible damage manifested by neuropathological changes in both neuronal and non-neuronal elements of the nervous system[1].

Nervous-system cell-type proteins as biochemical indicators of neurotoxicity

The nervous system is composed of highly differentiated cell-types with unique structural and functional characteristics. Nearly 2 decades ago, Moore[2] raised the possibility that the distinguishing qualities of nervous-system cell-types may be expressed by the presence of proteins specific to nervous tissue. Evidence in support of this concept has been obtained through recent advances in neuroimmunology and the widespread availability of methods for obtaining purified proteins from heterogeneous mixtures.

In both the developing and mature animal, specific soluble or membrane-bound proteins have been identified that are unique or characteristic of the diverse cellular and subcellular elements composing nervous tissue. To the cell biologist interested in the interaction of nervous-system cell-types grown in culture, nervous-system specific proteins represent markers with which to identify specific central and peripheral nervous-system cell-types. To the neurobiologist or neuropharmacologist concerned with the characterization of physiological functions associated with nervous-system cell-types, the properties of nervous-system specific proteins may reflect the function of individual cell-types. These and other applications of nervous-system specific proteins to neurobiological research have only recently been proposed[3].

Neurotoxicologists, like other neuroscientists, are also interested in nervous-system cell-types, since the unique properties of particular cell-types render them differentially susceptible to toxic insult. This cell-type specific action of toxic agents is widely documented on the basis of neuropathological data[1] and is often referred to as 'selective vulnerability'. Thus, toxicants are known to preferentially affect a given cell-type (e.g. neurons, oligodendroglia, etc.), or a given subclass of cell-type (e.g. specific types of neurons) often at the level of specific subcellular components (e.g. neuronal perikarya or synapses). Since these diverse targets of neurotoxic insult may be intimately linked with the unique biochemical composition of individual cell-types, nervous-system specific proteins can be exploited for evaluating the neurotoxic potential of broad classes of suspected toxic agents.

We propose that the detection and quantification of nervous system specific proteins following exposure to neurotoxicants may provide biochemical evidence indicative of irreversible or slowly reversible damage to the cellular elements that compose the nervous-system. Adoption of this neurotoxicity testing strategy would permit the characterization of cell-type reactions that are either the direct or indirect consequence of exposure of the developing or mature organism to putative neurotoxicants. In the mature organism changes in the levels of cell-type (marker) proteins would be indicative of damage to a particular cell-type, damage that, in the case of CNS neurons, is likely to be permanent. In the developing animal, changes in these proteins would reflect alterations in the ontogeny of processes critical to normal development such as neurogenesis, myelinogenesis and synaptogenesis.

In order to serve as indicators of neurotoxicity, nervous-system specific proteins should fulfil several criteria. Ideally, these would include the following:

(1) By definition, nervous-system specific proteins should be present only in nervous-tissue cell-types.

(2) They should be cell-type specific or at least enriched in specific cell-types, i.e. neurons and neuron types, oligodendroglia, astrocytes, etc.

(3) They should be specific to components of a particular cell-type, e.g. myelin sheath; nerve synapse, perikarya or axon, etc.

(4) Their ontogeny should be known and reflect processes susceptible to toxic insult (myelinogenesis, synaptogenesis, and neurogenesis, etc.).

(5) Levels or turnover of nervous-system specific proteins should not be significantly affected by variables that are often confounds in pharmacological research, e.g. sex, circadian rhythms, body temperature, etc.

Representative nervous-system specific proteins that meet most of these criteria are listed in Table I. With few exceptions, the proteins listed have been identified on the basis of both immunocytochemical tech-

niques and physiochemical properties. Although none have been exploited for the assessment of neurotoxicity, data exists that suggests that they may serve as useful indicators of toxicant-induced damage to nervous-system cell-types. For example, protein 1, the endogenous substrate for cyclic AMP- and calcium-dependent protein kinases[4], appears to be an excellent candidate as a marker for neurons. It is widely distributed in neurons of both the CNS and PNS, it is localized mainly to synaptic vesicles and its ontogeny coincides with synaptogenesis. Treatment of adult rats with the neurotoxicant, kainic acid, results in a dramatic loss of protein 1 from nervous tissue that coincides with the neuropathological changes produced by this compound.

Glial fibrillary acidic protein (GFAP) is a CNS protein specific for astrocytes[5]. Following injury or trauma to the CNS, astrocytes and their processes in the vicinity of the lesion undergo a vigorous response that includes extensive proliferation and hypertrophy[6]. Changes in GFAP faithfully reflect astrocytic reaction to injury[7] and this protein may therefore be an excellent marker for assessing astrocytic response to toxicant-induced damage to the central nervous system.

Proteins restricted to the myelin sheath and oligodendroglia that form myelin have also been characterized[8]. Treatment of experimental animals with a variety of structurally dissimilar compounds disrupts myelinogenesis or alters the integrity of the myelin sheath[1]. Many of these effects are accompanied by alterations in proteins specific to myelin. For example, we have found that exposure of the developing rat to neurotoxic organometals results in a persistent decrease in proteolipid and myelin basic proteins[9].

TABLE I. Examples of nervous-system specific proteins suitable for use as biochemical indicators of neurotoxicity

Tissue	Cell-type-component	Protein
CNS	Neurons	Synaptic membranes — Protein 1[4]
		Synaptic membranes — D2[14]
		Synaptic membranes — B50[19]
		Synaptic vesicles — Protein 1[4]
		Neurites — D2[14]
		Postsynaptic density — PSD 95[20]
	Glia	Oligodendroglia — Myelin basic protein[8]
		Oligodendroglia — Myelin associated glycoprotein[8]
		Myelin sheath — Myelin basic protein[8]
		Fibrous astrocytes — Glial fibrillary acidic protein[5]
PNS	Neurons	Synapse — Protein 1[4]
		Neurites — D2[14]
	Schwann cell	Myelin sheath — PO[9]

Lessons from clinical chemistry

The use of biochemical markers as indices of cell damage (including neuronal) is not unprecedented. This concept has been broadly applied in the field of clinical chemistry. The modern-day clinical chemist possesses a broad armamentarium of biochemical indicators with which to diagnose pathological changes in specific organ systems[10]. For example, widely distributed intracellular enzymes, such as lactic dehydrogenase (LDH) and creatinine phosphokinase (CPK) are used as indicators of pathological conditions. The detection of these enzymes in serum or plasma is widely accepted as an indicator of cell death or increased cellular permeability associated with specific disease states[10].

Although readily accessible biological fluids, such as blood and urine, can be used as sources of markers pathognomonic of visceral damage, the use of these fluids for the detection of indicators of brain damage is rare, since the brain lacks direct exchange with the blood due to the presence of the blood–brain barrier. However, because the CSF is in direct contact with the brain, one might expect that pathological changes in the CNS would be reflected in the CSF. Indeed, most efforts to develop clinical indicators of neuropathology have focused on the CSF[11]. For example, elevated levels of LDH and CPK in the CSF have been employed as indicators of CNS cell death[11], unfortunately, measurements of these enzymes will not distinguish the affected cell-type. Furthermore, since a compromised blood–brain barrier may be one result of toxicant exposure, the appearance of general indicators of cell death in the CSF may reflect damage to organs or tissues other than the nervous system. Although the detection and quantification of nervous-system specific proteins in the CSF would appear to offer a solution to this dilemma, only a few examples exist where this approach has been applied to the assessment of CNS damage.

Perhaps the best example of a biochemical marker that has been used to diagnose damage to a specific brain component is the myelin-specific protein, myelin basic protein. Patients with multiple sclerosis undergoing active attacks have been found to release myelin basic protein into the CSF, whereas patients with inactive multiple sclerosis do not have significant levels of this protein in CSF[12].

Markers of other nervous-system cell-types have also been identified in the CSF. For example, GFAP, the astrocyte marker, has been detected in the CSF by radioimmunoassay and may serve as a useful marker in the diagnosis of specific types of gliomas[13]. The synaptic membrane protein, D2, a neuron-specific protein[14], has been detected not only in CSF[15], but also in amniotic fluid[16]. D2 has been used in prenatal diagnosis to detect neural tube defects, and in adult CSF it may be indicative of certain neuropathological states.

Based on these clinical findings, it seems possible that the measurement of nervous-system specific proteins may be applied to the CSF for the detection of neurotoxic responses. If nervous-system specific proteins can be detected in the CSF of experimental animals following exposure to neurotoxicants, then these data may be used to augment measurements of nervous-system specific proteins in CNS tissue.

Future directions: a role for monoclonal antibodies

The advent of the hybridoma technique for producing monoclonal antibodies[17] should herald a new era in cell-type marker methodology. These antibodies can be continuously supplied and have the advantage of being directed toward a single antigenic determinant, even when derived from crude sources of antigenic material.

Application of monoclonal methodology to neurobiology has already resulted in the discovery of several novel nervous-system cell-type antigens[18]. In the future, the use of this technique should result in the detection (through immunocytohistochemical techniques) and quantification (through the use of monoclonal-based radioimmunoassays)

of cell-type specific proteins distributed throughout the nervous system. This advance in technology should aid in the validation of nervous-system specific proteins as biochemical indicators of neurotoxicity.

Note

This paper has been reviewed by the Health Effects Research Laboratory, US Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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